

Ca 23 S AFNTQAAP 31
Cs 1 G 1

Tr2#		10	20	30	40	
Bp	1	RTITNEMGN	HSGYDYELWK	DYGNT-SMTL	NNGGAFSAGW	N--NIGNA 45
Ca	32	KTITSNEIGV	NGGYDYELWK	DYGNT-SMTL	KNGGAFSCQW	S--NIGNA 76
Fs	1	NSSVTGNVG	SSPYHYEIIWY	QGG-NNSMTF	YDNGTYKASW	N--GTNDF 44
Cs	2	RRIYDNETGT	HGGYDYELWK	DYGNT-IMEL	NDGGTFSCQW	S--NIGNA 46
Rf	1	SAADQQTRGN	VGGYDYEMMN	QNGQQQASMN	PGAGSFTCSW	S--NIENF 46
Tr2	1	QTIQPGTGY	NNGYFYSYWN	DGHGGVITYN	GPCGGQFSVNW	S--NSGNF 45
Tv	1	QTIQPGTGF	NNGYFYSYWN	DGHGGVITYN	GPCGGQFSVNW	S--NSGNF 45
Th	1	QTIQPGTGY	SNGYYSYWN	DGHAGVITYN	GGGGSFVNW	S--NSGNF 45
Sc	1	SGTPSSTGT	DGGVYYSWWT	DGAGDATYQN	NGGGSYTLTH	SG--NNGNL 46
An	1		S AGINYVQNYN	GNLGDFTY-D	ESAGTFSMYW	EDGVSSDF 38
Ak	1		S AGINYVQNYN	GNLADFTY-D	ESAGTFSMYW	EDGVSSDF 38
AT	1		S AGINYVQNYN	GNLGDFTY-D	ESAGTFSMYW	EDGVSSDF 38
Tr1	1		ASINYDQNYQ	TGG-QVSYS-	PSNTGFSVNW	N--TQDDF 34
Aa	1	RSTPSSTGE	NNGYYSFWT	DGGGDVITYN	GNAGSYSVW	S--NVGNF 45
Ss	1	ATTIT-NETGY	D-GMYYSFWT	DGGGSVSMTL	NGGGSYSTRW	T--NCGNF 45
SLB	1	DTVVTTNQEQT	NNGYYSFWT	DSQCTVSMNM	GSQGQYSTSW	R--NTGNF 47
SLC	1	ATTITNTQTGT	D-GMYYSFWT	DGGGSVSMTL	NGGGSYSTQW	T--NCGNF 46
Tl	1	QTTPNSEGW	HDGYYSWWS	DGGAQATYTN	LEGGTYSISW	G--DGGNL 45
Tf	1	AVTSNETGY	HDGYYSFWT	DAGTVSMEL	GPCGNYSTSW	R--NTGNF 45
Bc	1		ASTDYWQNW	DGGGIVNAVN	GSGGNYSVNW	S--NTGNF 36
Bs	1		ASTDYWQNW	DGGGIVNAVN	GSGGNYSVNW	S--NTGNF 36

FIGURE 1

09900874-12104

Tr2#		50		60		70		80	
Bp	46	LFRK-GKKFD	ST-RTHHQLG	NISINYNASF	N-PSGNSYLC	VYGTQSP		90	
Ca	77	LFRK-GKKPN	DT-QTYKQLG	NISVNYNCNY	Q-PYGNLYLC	VYGTSSP		121	
FS	45	LARV-GFKYD	EK-HTYEELGP	IDAYYKWSKQ	GSAGGYNYIG	LYGTVDP		91	
Cs	47	LFRK-GRKFN	SD-KTYQBLG	DIUVEYGCXY	N-PNGNSYLC	VYGTTRNF		91	
Rf	47	LARM-GKNYD	SQKKNYKAFG	NIUVTYDVEY	T-PRGNSYMC	VYGTTRNP		92	
Tr2	46	VGGK-GWQPG	TKNKV----	---INFS-GS	YNPNNGNSYLS	VYGTSRNP		83	
Tv	46	VGGK-GWQPG	TKNKV----	---INFS-GS	YNPNNGNSYLS	VYGTSRNP		83	
Th	46	VGGK-GWQPG	TKNKV----	---INFS-GS	YNPNNGNSYLS	VYGTSRNP		83	
Sc	47	VGGK-GWNP	AASRS----	---ISYS-GT	YQPNNGNSYLS	VYGTTRSS		84	
An	39	VVGL-GWTTG	SSNA-----	---ITYSAEY	SASGSSSYLA	VYGVVNYF		77	
Ak	39	VVGL-GWTTG	SSNA-----	---ITYSAEY	SASGSSSYLA	VYGVVNYF		77	
At	39	VVGL-GWTTG	SSNA-----	---ITYSAEY	SASGSSSYLA	VYGVVNYF		77	
Tr1	35	VVGW-GWTTG	SSAP-----	---INFGCSF	SVNSGTGLLS	VYGTSTNP		72	
Aa	46	VGGK-GWNP	SAKD-----	---ITYSGNF	T-PSGNSYLS	VYGTTPD		83	
Sa	46	VAGK-GWANG	GR-RT-----	---VRYT-GW	FNPNSGNYGC	LYGWTSP		82	
SLB	48	VAGK-GWANG	GR-RT-----	---VQYS-GS	FNPNSGNYLA	LYGWTSP		84	
SLC	47	VAGK-GWSTG	DGN-----	---VRYN-GV	FNPVNGYGC	LYGWTSP		82	
TL	46	VGGK-GWNP	LNARA-----	---IHFE-GV	YQPNNGNSYLA	VYGTTRNP		83	
Tf	46	VAGK-GWATG	GR-RT-----	---VTYS-AS	FNPNSGNYLT	LYGTTRNP		82	
Bc	37	VVGK-GWTTG	SPFRT-----	---INYNAGV	WAPNGNGYLT	LYGTTRSP		75	
Bs	37	VVGK-GWTTG	SPFRT-----	---INYNAGV	WAPNGNGYLT	LYGTTRSP		75	

Tr2#		90		100		110		120		130
Bp	91	LAEYIVDSW	GTyr-PT--G	AYKGSFYADG	GTyDIYETTR	VNOPSIG		135		
Ca	122	LVEYIVDSW	GSWRPP--GG	TSKGTITVDG	GIYDIYETTR	INOPSIG		167		
FS	92	LVEYIVDDW	FNKPGANLLG	QRKGFTVDG	DTYEIWQNT	VQOPSIG		139		
Cs	92	LVEYIVESW	GSWRPP--GA	TPKGTITQWMA	GTyDIYETTR	VNOPSIG		138		
Rf	93	LMYIVESW	GDWRPPGNDG	EVKGTVSANG	NTYDIRKTMR	VNOPSIG		140		
Tr2	84	LIEYIVENF	GTYN-PTGA	TKLGEVTSDG	SVYDIYRTQR	VNOPSIG		130		
Tv	84	LIEYIVENF	GTYN-PTGA	TKLGEVTSDG	SVYDIYRTQR	VNOPSIG		130		
Th	84	LIEYIVENF	GTYN-PTGA	TKLGEVTSDG	SVYDIYRTQR	VNOPSIG		130		
Sc	85	LIEYIVESY	GSYD-PSSAA	SHKGSVTCNG	ATYDILSTWR	VNOPSIG		131		
An	78	GAEYIVEDY	GDYN-PCSSA	TSLGTVYSDG	STYQVCIDTR	INEPSIG		124		
Ak	78	QAEYIVEDY	GDYN-PCSSA	TSLGTVYSDG	STYQVCIDTR	INEPSIG		124		
At	78	QAEYIVEDY	GDYN-PCSSA	TSLGTVYSDG	STYQVCIDTR	INEPSIG		124		
Tr1	73	LVEYIVMEDN	HNY--PAQ-G	TVKGTVTSDD	ATYTIWENTR	VNOPSIG		117		
Aa	84	LIEYIVESY	GSYD-PGSGG	TTKGNVSSDG	SVYDIYATR	TNAPSIG		130		
Sa	83	LVEYIVDNW	GSYR-PT--G	ETRGTVHSDG	GTyDIYKTR	VNAPSIG		127		
SLB	85	LVEYIVDNW	GSYR-PT--G	EYKGTVTSDD	GTyDIYKTR	VNAPSIG		129		
SLC	83	LVEYIVDNW	GSYR-PT--G	TVKGTVSSDG	GTyDIYKTR	VNAPSIG		127		
TL	84	LVEYIVENF	GTyD-PSSGA	TDLGTVECDG	STYRLKTR	VNAPSIG		130		
Tf	83	LVEYIVESY	GTyD-PT--G	TYMGTVTDDG	GTyDIYKTR	VNAPSIG		127		
Bc	76	LIEYIVDSW	GTyR-PT--G	TYKGTVKSDG	GTyDIYITR	VNAPSIG		120		
Bs	76	LIEYIVDSW	GTyR-PT--G	TYKGTVKSDG	GTyDIYITR	VNAPSIG		120		

FIGURE 1 CONT'D

Tr2#		140	150	160		
Bp 136	-LATEFQYWS	VRQTKRST--	-----GTVS	VSAHPRKWS	LGMPM-GK 174	
Ca 168	-NTTEFQYWS	VRQTKRST--	-----GTIS	VSKHFAAWES	KGMLP-GK 206	
Fs 140	-TQTFEQYFS	VRKSARSC--	-----GHID	ITAHMKKWE	LGMMK-GK 178	
Cs 139	-TATEFQYWS	VRTSKRTS--	-----GTIS	VTEHFKQWER	MGMRM-GK 177	
Rf 141	-TATEFQYWS	VRQTSQSANN	QTNVMKGTID	VSKHFDWASA	AGLDMSGT 187	
Tr2 131	-TATEFQYWS	VRNHR-S-S	-----GSVN	TANHFNAWAQ	QGLTL-GT 168	
Tv 131	-TATEFQYWS	VRTHR-S-S	-----GSVN	TANHFNAWAQ	QGLTL-GT 168	
Th 131	-TATEFQYWS	VRNHR-S-S	-----GSVN	TANHFNAWAS	HGLTL-GT 168	
Sc 132	-TQTFEQYWS	VRNPKKAPGG	SIS---	GTVD	VQCHFDWAKG	LGMLGSE 175
An 125	-TSTFQYFS	VRSTRSTS--	-----GTVT	VANHFNFWAQ	HGFNG-SD 163	
Ak 125	-TSTFQYFS	VRSTRSTS--	-----GTVT	VANHFNFWAQ	HGFNG-SD 163	
At 125	-TSTFQYFS	VRSTRSTS--	-----GTVT	VANHFNFWAH	HGFHN-SD 163	
Tr1 118	-TATFNQYIS	VRNSPR-T-S	-----GTVT	VQNHFN-WAS	LGLHLGQM 155	
Aa 131	-TQTFEQYWS	VRQNK-R-VG	-----GTVT	TSNHFNAWAK	LGMLL-GT 168	
Ss 128	-PAAPDQYWS	VRQSKVT--S	-----GTIT	TGNHFDAWAR	AGMNMGNF 168	
SlB 130	TR-TQDQYWS	VRQSKR-TG-	-----GTIT	TGNHFDAWAR	AGMFLGNF 168	
SlC 128	TK-TQDQYWS	VRQSKVTSGS	-----GTIT	TGNHFDAWAR	AGMNMGNF 168	
Tl 131	TQ-TQDQYWS	VRQDKR-T-S	-----GTQV	TGCHFDWAKR	AGLNVNGD 169	
Tf 128	TR-TQDQYWS	VRQSKRTS--	-----GTIT	AGNHFDAWAR	HGMHLGTH 166	
Bc 121	DRTFEQYWS	VRQSKRPTGS	N-----ATIT	FTNHVNAWKS	HGMNLGSN 163	
Bs 121	DRTFEQYWS	VRQSKRPTGS	N-----ATIT	FSNHVNAWKS	HGMNLGSN 163	

Tr2#		170	180	190	
Bp 175	MYETAFTVGG	YQSSGGSANVM	TNQLFIGN		201
Ca 207	MHETAFNIEG	YQSSGKADVN	SMSINIGK		233
Fs 179	MYEAKVLVEA	GGGSGSFDV-	TYFKMT		203
Cs 178	MYEVALTVEG	YQSSGYANVY	KNEIRIGANP....		
Rf 188	LYEVSINIIEG	YRSNGSANVK	SVSV		211
Tr2 169	MDYQIVAVEG	YFSSGGSASI-	TVS		190
Tv 169	MDYQIVAVEG	YFSSGGSASI-	TVS		190
Th 169	MDYQIVAVEG	YFSSGGSASI-	TVS		190
Sc 176	HNQIVATEG	YQSSGTATI-	TVT		197
An 164	FNQVMAVEA	WSGAGSASV-	TISS		185
Ak 164	FNQVMAVEA	WSGAGSASV-	TISS		185
At 164	FNQVMAVEA	WSGAGSAAV-	TISS		185
Tr1 157	MNYQVVAVEG	WGGSGGSASQ-	SVSN		178
Aa 169	HNQIILATEG	YQSSGSSSI-	TIQ		190
Ss 167	RYYMINATEG	YQSSGSSSTI-	TVSG		169
SlB 169	SYYMINATEG	YQSSGTSSI-	NVGG.....		
SlC 169	RYYMINATEG	YQSSGSSNI-	TVSG		191
Tl 170	HYQIVATEG	YFSSGYARI-	TVADVG		194
Tf 167	D-YMINATEG	YQSSGSSNVT	LOTS.....		
Bc 164	WAYQVMAVEG	YQSSGSSNV-	TVW		185
Bs 164	WAYQVMAVEG	YQSSGSSNV-	TVW		185

FIGURE 1 CONT'D

Bp *Bacillus pumilus*
Ca *Clostridium acetobutylicum* P262 XynB
Cs *Clostridium stercoararium* xynA
Rf *Ruminococcus flavefaciens*
Tr2 *Trichoderma reesei* XYN II
Tv *Trichoderma viride*
Th *Trichoderma harzianum*
Sc *Schizophyllum commune* Xylanase A
An *Aspergillus niger*, var. *awamori*
Ak *Aspergillus kawachii* XynC
At *Aspergillus tubigensis*
Tr1 *Trichoderma reesei* XYN I
Aa *Aspergillus awamori* var. *kawachi* Xyn B
Fs *Fibrobacter sccinogenes* XYN II
Ss *Streptomyces* sp. 36a
SlB *Streptomyces lividans* Xln B
SlC *Streptomyces lividans* Xln C
Tl *Thermomyces lanuginosus* Xyn
Tf *Thermomonospora fusca* TfxA
Bc *Bacillus circulans*
Bs *Bacillus subtilis*

FIGURE 1 CONT'D

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st

5'-CT AGC TAA GGA GG CTG CAG ATG
G ATT CCT CC GAC GTC TAC
 NheI PstI

TrX-1

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Q	T	I	Q	P	G	T	G	Y	N	N	G	Y	F	Y	S
CAA	ACA	ATA	CAA	CCA	GGA	ACC	GGT	TAC	AAC	AAC	GGT	TAC	TTT	TAC	AGC
GTT	TGT	TAT	GTT	GGT	CCT	<u>TGG CCA</u>	ATG	TTG	TTG	CCA	ATG	AAA	ATG	TCG	

TrX-8 AgeI

XyTv-2

17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Y	W	N	D	G	H	G	G	V	T	Y	T	N	G	P	G
TAT	TGG	AAC	GAT	GGC	CAT	GGT	GGT	GTT	ACC	TAT	ACA	AAC	GGG	CCC	GGA
ATA	ACC	TTG	CTA	CCG	<u>GTA CCA</u>	CCA	CAA	TGG	ATA	TGT	TTG	<u>CCC GGG</u>	CCT		

NcoI XyTv-7 ApaI

33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
G	Q	F	S	V	N	W	S	N	S	G	N	F	V	G	G
GGC	CAA	TTT	AGC	GTC	AAT	TGG	TCT	AAC	TCC	GGA	AAC	TTC	GTA	GGT	GGA
CCG	GTT	AAA	TCG	CAG	<u>TTA ACC</u>	AGA	TTG	<u>AGG CCT</u>	TTG	AAG	CAT	CCA	CCT		

MunI BspEI

TrX-3

49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64
K	G	W	Q	P	G	T	K	N	K	V	I	N	F	S	G
AAA	GGT	TGG	CAA	CCC	GGG	ACC	AAA	AAT	AAG	GTG	ATC	AAC	TTC	TCT	GGA
TTT	CCA	ACC	GTT	<u>GGG CCC</u>	TGG	TTT	TTA	TTC	CAC	TAG	TTG	AAG	AGA	CCT	

XmaI TrX-6

65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
S	Y	N	P	N	G	N	S	Y	L	S	V	Y	G	W	S
TCT	TAT	AAT	CCG	AAT	GGG	AAT	TCA	TAC	TTA	AGC	GTC	TAT	GGC	TGG	TCT
AGA	ATA	TTA	GGC	TTA	<u>CCC TTA AGT</u>	ATG	<u>AAT TCG</u>	CAG	ATA	CCG	ACC	<u>AGA</u>			

EcoRI AflII

XyTv-4

81	82	83	84	85	86	87	88	89	90	91	92	93	94	95
R	N	P	L	I	E	Y	Y	I	V	E	N	F	G	T
AGA	AAC	CCA	CTG	ATT	GAA	TAT	TAC	ATT	GTC	GAA	AAT	TTC	GGT	AC
<u>TCT</u>	TTG	GGT	GAC	TAA	CTT	ATA	ATG	TAA	CAG	CTT	TTA	AAG	<u>C</u>	

Xba I XyTv-5 KpnI

FIGURE 2

XyTv-101

V	D	92	93	94	95	96	97	98	99	100	101	102	103	104	105
TC	GAC	AAT	TTC	GGT	ACC	TAC	AAT	CCG	AGT	ACC	GGC	GCC	ACA	AAA	TTA
3'-G	TTA	AAG	<u>CCA</u>	<u>TGG</u>	ATG	TTA	GGC	TCA	TGG	<u>CCG</u>	<u>CGG</u>	TGT	TTT	AAT	
<u>SalI</u>			<u>KpnI</u>					<u>XyTv-110</u>		<u>KasI/NarI</u>					

XyTv-102

106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121
G	E	V	T	S	D	G	S	V	Y	D	I	Y	R	T	Q
GGC	GAA	GTC	ACT	AGT	GAT	GGA	TCC	GTA	TAT	GAT	ATC	TAC	CGT	ACC	CAA
CCG	CTT	CAG	<u>TGA</u>	<u>TCA</u>	CTA	<u>CCT</u>	<u>AGG</u>	CAT	ATA	CTA	TAG	ATG	GCA	TGG	GTT
			<u>SpeI</u>			<u>BamHI</u>							<u>XyTv-109</u>		

Trx-103

122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137
R	V	N	Q	P	S	I	I	G	T	A	T	F	Y	Q	Y
CGC	GTT	AAT	CAG	CCA	TGC	ATC	ATT	GGA	ACC	GCC	ACC	TTT	TAT	CAG	TAC
<u>CGC</u>	<u>CAA</u>	<u>TTA</u>	<u>GTC</u>	<u>GGT</u>	<u>AGC</u>	<u>TAG</u>	<u>TAA</u>	<u>CCT</u>	<u>TGG</u>	<u>CGG</u>	<u>TGG</u>	<u>AAA</u>	<u>ATA</u>	<u>GTC</u>	<u>ATG</u>
<u>MluI</u>				<u>Clai</u>											

138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153
W	S	V	R	R	N	H	R	S	S	G	S	V	N	T	A
TGG	AGT	GTT	AGA	CGT	AAT	CAT	CGG	AGC	TCC	GGT	TGC	GTT	AAT	ACT	GCG
ACC	TCA	CAA	TCT	GCA	TTA	GTA	<u>GCC</u>	<u>TGC</u>	<u>AGG</u>	CCA	AGC	CAA	TTA	TGA	CGC
	<u>Trx-108</u>							<u>SacI</u>							

XyTv-104

154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169
N	H	F	N	A	W	A	Q	Q	G	L	T	L	G	T	M
AAT	CAC	TTT	AAT	GCA	TGG	GCA	CAG	CAA	GGG	TTA	ACC	CTA	GGT	ACA	ATG
TTA	GTG	AAA	<u>TTA</u>	<u>CGT</u>	<u>ACC</u>	CGT	GTC	GTT	CCC	AAT	<u>TGG</u>	<u>GAT</u>	<u>CCA</u>	TGT	TAC
			<u>NsiI</u>			<u>XyTv-107</u>				<u>AvrII</u>					

XyTv-105

170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185
D	Y	Q	I	V	A	V	E	G	Y	F	S	S	G	S	A
GAT	TAT	CAA	ATC	GTA	GCG	GTG	GAA	GGC	TAC	TTC	TGC	AGT	GGT	TCC	GCT
CTA	ATA	GTT	TAG	CAT	CGC	CAC	CTT	CCG	ATG	<u>AAG</u>	<u>AGC</u>	<u>TCA</u>	<u>CCA</u>	<u>AGG</u>	<u>CGA</u>
						<u>XyTv-106</u>				<u>XhoI</u>					

186	187	188	189	190
S	I	T	V	S
AGT	ATT	ACA	GTG	AGC
TCA	TAA	TGT	CAC	TCG
				<u>TCT</u>
				<u>BglII</u>

FIGURE 2 CONT'D

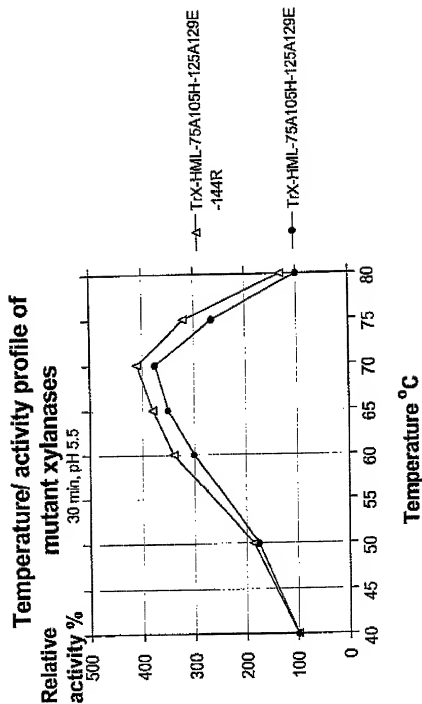
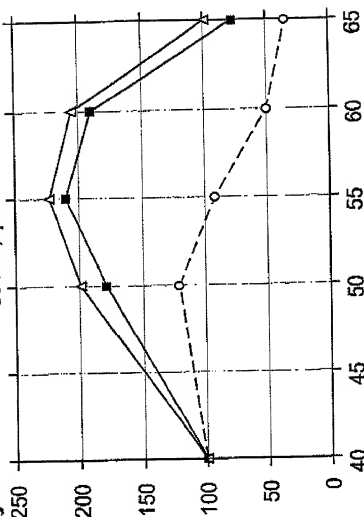


Figure 3

Temperature /activity profile of mutant xylanases

30 min, pH 5

Relative
activity %



—△— TrX-116G

—■— TrX-118C

---○--- TrX

Temperature °C

Figure 4

Effect of Temperature on the activity of mutant xylanase

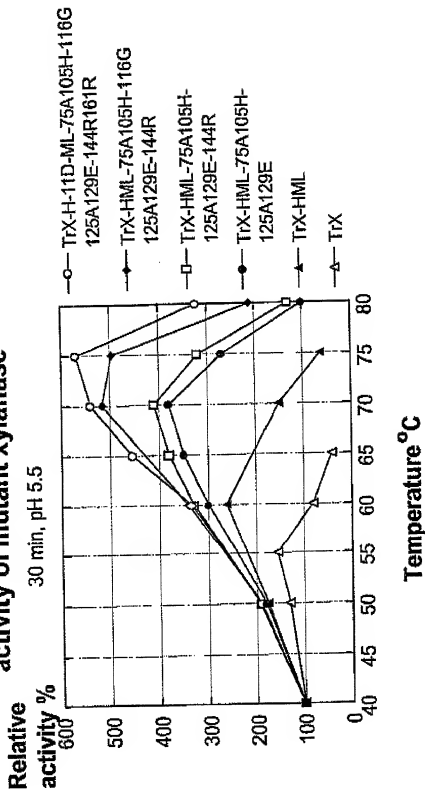


Figure 5

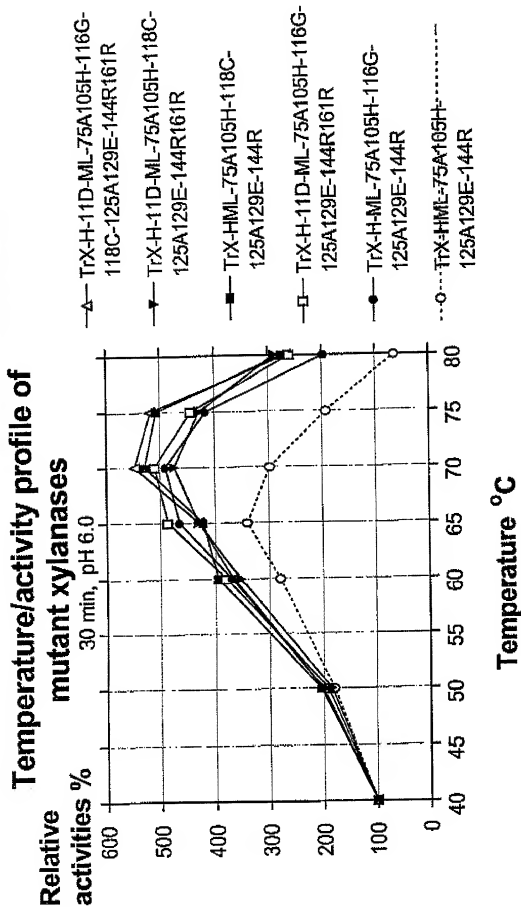


Figure 6

Temperature/activity profile of mutant xylanases

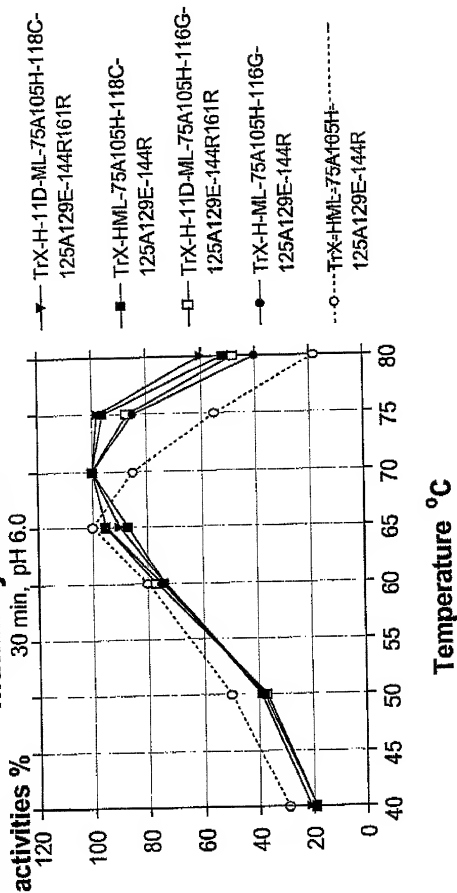


Figure 7

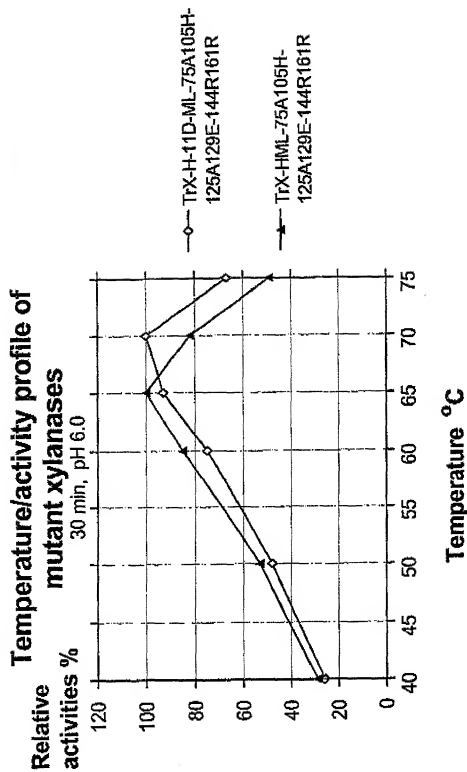


Figure 8

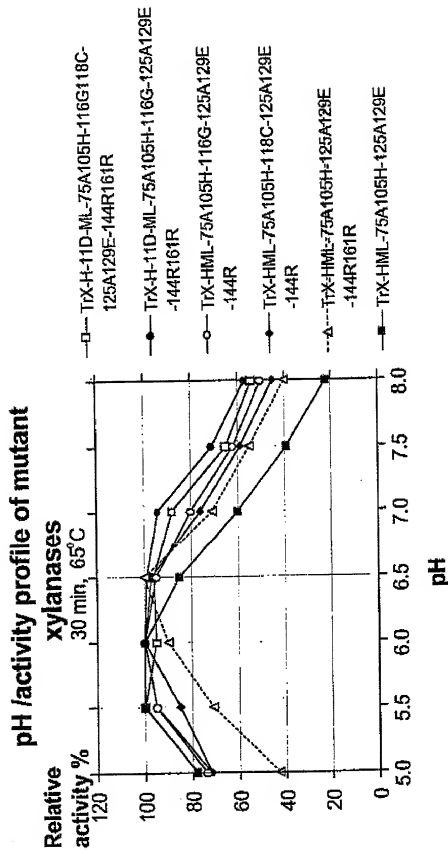


Figure 9

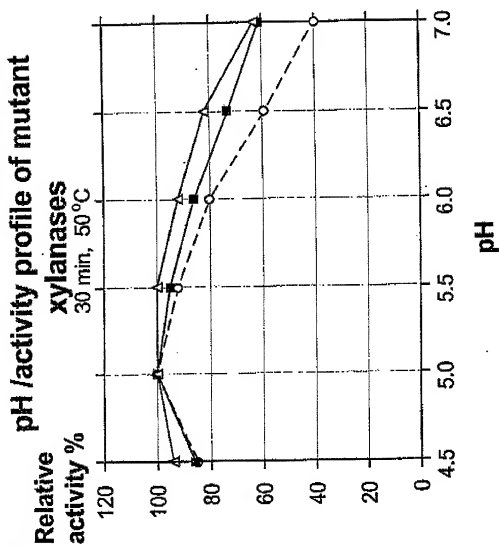


Figure 10

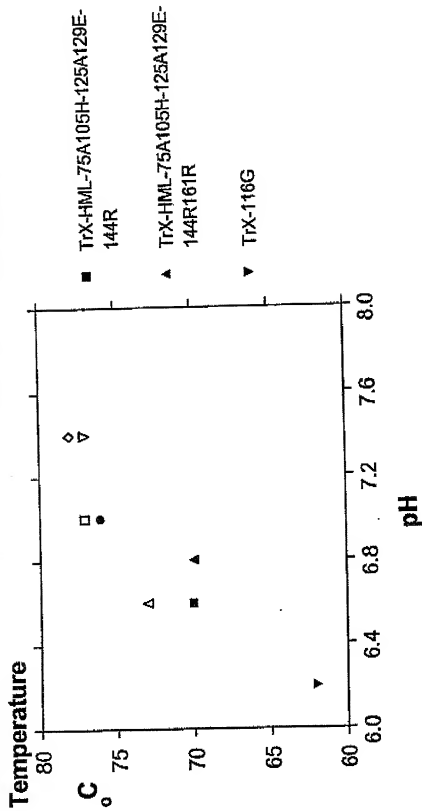


Figure 11

- TrX-HML-75A105H-116G-125A129E-144R
- TrX-HML-75A105H-118C-125A129E-144R
- ▲ TrX-H-11D-ML-75A105H-125A129E-144R161R
- ▼ TrX-H-11D-ML-75A105H-116G-125A129E-144R161R
- ◇ TrX-H-11D-ML-75A105H-118C-125A129E-144R161R